Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon

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Abstract: OBJECTIVES The aim of this study was to describe clinical and virological outcomes in therapy-naive HIV-1-positive patients treated in a routine ART programme in rural Cameroon. METHODS In a prospective cohort, 300 consecutive patients starting first-line ART were enrolled and followed for 12 months. Among 238 patients with available viral load data at Month 12, logistic regression was used to analyse risk factors for virological failure (1000 HIV RNA copies/mL) including clinical, immunological and virological parameters, as well as data on drug adherence. Population sequencing was performed to detect the presence of drug-resistance mutations in patients with virological failure at Month 12; minority drug-resistance mutations at baseline were analysed using next-generation sequencing in these patients and matched controls. RESULTS At Month 12, 38/238 (16%) patients experienced virological failure (1000 HIV RNA copies/mL). Patients with virological failure were younger, had lower CD4 cell counts and were more often WHO stage 3 or 4 at baseline. Sixty-three percent of patients with virological failure developed at least one drug-resistance mutation. The M184V (n = 18) and K103N (n = 10) mutations were most common. At baseline, 6/30 patients (20%) experiencing virological failure and 6/35 (17%) matched controls had evidence of minority drug-resistance mutations using next-generation sequencing (P = 0.77). Lower CD4 count at baseline (OR per 100 cells/mm(3) lower 1.41, 95% CI 1.02-1.96, P = 0.04) and poorer adherence (OR per 1% lower 1.05, 95% CI 1.02-1.08, P < 0.001) were associated with a higher risk of virological failure. Unavailability of ART at the treatment centre was the single most common cause for incomplete adherence. CONCLUSIONS Virological failure after 1 year of ART was not associated with minority drug resistance at baseline but with incomplete adherence. Strategies to assure adherence and uninterrupted drug supplies are pivotal factors for therapy success.

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Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon

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Objectives: The aim of this study was to describe clinical and virological outcomes in therapy-naive HIV-1-positive patients treated in a routine ART programme in rural Cameroon.

Methods: In a prospective cohort, 300 consecutive patients starting first-line ART were enrolled and followed for 12 months. Among 238 patients with available viral load data at Month 12, logistic regression was used to analyse risk factors for virological failure (≥1000 HIV RNA copies/mL) including clinical, immunological and virological parameters, as well as data on drug adherence. Population sequencing was performed to detect the presence of drug-resistance mutations in patients with virological failure at Month 12; minority drug-resistance mutations at baseline were analysed using next-generation sequencing in these patients and matched controls.

Results: At Month 12, 38/238 (16%) patients experienced virological failure (≥1000 HIV RNA copies/mL). Patients with virological failure were younger, had lower CD4 cell counts and were more often WHO stage 3 or 4 at baseline. Sixty-three percent of patients with virological failure developed at least one drug-resistance mutation. The M184V ($n=18$) and K103N ($n=10$) mutations were most common. At baseline, 6/30 patients (20%) experiencing virological failure and 6/35 (17%) matched controls had evidence of minority drug-resistance mutations using next-generation sequencing ($P=0.77$). Lower CD4 count at baseline (OR per 100 cells/mm^3 lower 1.41, 95% CI 1.02–1.96, $P=0.04$) and poorer adherence (OR per 1% lower 1.05, 95% CI 1.02–1.08, $P<0.001$) were associated with a higher risk of virological failure. Unavailability of ART at the treatment centre was the single most common cause for incomplete adherence.

Conclusions: Virological failure after 1 year of ART was not associated with minority drug resistance at baseline but with incomplete adherence. Strategies to assure adherence and uninterrupted drug supplies are pivotal factors for therapy success.

Keywords: HIV drug resistance, adherence, Africa

Introduction

The scaling up of antiretroviral treatment programmes in sub-Saharan Africa has led to favourable results.[1,2] However, poor retention in care,[3] inadequate adherence to treatment and lack of stock of antiretroviral drugs among others have been identified as factors contributing to adverse outcomes of ART and the development of drug resistance.[3,4] Furthermore, it has been shown that up to 7.4% of antiretroviral-naive patients harbour a primary drug-resistant virus, although data from central Africa are scarce.[5] In the present prospective cohort study, we aimed to assess the prevalence and determinants of virological failure, including primary drug resistance, in HIV-infected patients in Cameroon.

Methods

In the outpatient HIV clinic of the Bamenda Regional Hospital, patients were assessed for ART eligibility according to national guidelines and WHO recommendations.[6] At the time of the study, two doctors together

with pharmacists and social workers were attending to a total of 4500 HIV patients and more than 30 new patients every month. Ethical approval for the performance of the study was granted by the National Ethics Committee in Yaounde, Cameroon. Written informed consent was obtained from all of the individuals.

From January 2010 to October 2010, 300 consecutive patients being initiated on first-line ART were included in the study. The sample size was determined assuming a prevalence of antiretroviral resistance of 20%, a precision of 5% and a drop-out rate of 20%. Patients were monitored every 3 months by a study physician. Every 6 months, an analysis of blood count, liver function tests and CD4+ T cell count was performed and plasma was cryopreserved. The returned pills were counted and the adherence ratio was calculated as the number of days a patient was taking the correct number of prescribed antiretroviral drugs divided by the number of days since the start of therapy.

Viral load testing was retrospectively performed using the Abbott real-time HIV-1 amplification reagent kit (University of Bonn). Drug-resistance testing in patients virologically failing at Month 12 by population sequencing was performed using the Viroseq version 2 (Abbott) (University of Zurich). The retrospective drug-resistance testing of baseline samples by next-generation sequencing was performed using 454 pyrosequencing technology as previously described[7] (University of Zurich; see the Supplementary data available at *JAC* Online for details) on patients with virological failure at Month 12 and an equal number of patients

Table 1. Baseline characteristics of patients with available viral load (VL) data at Month 12 ($n=238$)

	Virological success (VL <1000 HIV RNA copies/mL, $n=200$)	Virological failure (VL ≥1000 HIV RNA copies/mL, $n=38$)	*P*
Gender, *n* (%)			0.07[a]
male	60 (30)	9 (24)	
female	140 (70)	29 (76)	
Education, *n* (%)			0.29[a]
none	6 (3)	1 (3)	
primary	111 (56)	22 (58)	
secondary	74 (37)	13 (34)	
university/higher	9 (5)	2 (5)	
History of TB, *n* (%)			0.10[a]
no	179 (90)	31 (82)	
yes	21 (11)	6 (16)	
Current TB, *n* (%)			0.12[a]
no	191 (96)	35 (92)	
yes	9 (5)	2 (5)	
WHO stage, *n* (%)			0.01[a]
1	94 (47)	12 (32)	
2	50 (25)	10 (26)	
3	40 (20)	9 (24)	
4	15 (8)	5 (13)	
NRTI backbone, *n* (%)			0.35[a]
ZDV/3TC	165 (83)	28 (74)	
d4T/3TC	2 (1)	0 (0)	
TDF/3TC	13 (7)	6 (16)	
TDF/FTC	20 (10)	4 (11)	
Third drug, *n* (%)			0.92[a]
ABC	1 (1)	0 (0)	
EFV	49 (25)	10 (26)	
LPV/r	9 (5)	1 (3)	
NVP	141 (71)	27 (71)	
Age (years), median (range)	37.8 (19.5–78.1)	34.9 (22.3–79.5)	0.03[b]
CD4+ T cells/mm^3, median (range)	184 (1–889)	116 (3–349)	0.05[b]
VL ($\log_{10}$ HIV RNA copies/mL), median (range)	5.4 (2.3–6.7)	5.5 (3.3–6.4)	0.99[b]
Haemoglobin (mg/dL), median (range)	11 (6.3–20.3)	10.7 (5.6–17)	0.54[b]

ZDV, zidovudine; 3TC, lamivudine; d4T, stavudine; TDF, tenofovir; FTC, emtricitabine; ABC, abacavir; EFV, efavirenz; LPV/r, lopinavir boosted with ritonavir; NVP, nevirapine.
[a]χ^2 test.
[b]Wilcoxon rank sum test.

Table 2. Univariable and multivariable logistic regression analysing risk factors for virological failure

	Univariable logistic regression			Multivariable logistic regression		
	OR	95% CI	*P*>z	OR	95% CI	*P*>z
Female sex (versus male)	1.33	0.59–3	0.49			
Age (per 10 years older)	0.71	0.49–1.02	0.07	0.79	0.54–1.15	0.21
CD4 baseline (per 100 cells/mm^3 lower)	1.41	1.02–1.96	0.04	1.47	1.02–2.08	0.04
WHO Stage 3 or 4 (yes versus no)	1.96	0.95–4.02	0.07	1.38	0.61–3.11	0.43
Viral load at baseline (per 1 $\log_{10}$ HIV RNA copies/mL higher)	1.06	0.64–1.77	0.81			
Efavirenz at baseline (yes versus no)	1.24	0.56–2.77	0.59			
Nevirapine at baseline (yes versus no)	0.96	0.44–2.07	0.91			
Current TB (yes versus no)	1.25	0.26–6.03	0.78			
Haemoglobin at baseline (per 1 mg/dL higher)	0.91	0.76–1.09	0.33			
Adherence ratio (per 1% lower)[a]	1.05	1.02–1.08	<0.001	1.04	1.02–1.07	<0.001

[a]From baseline to last clinical follow-up.

with a viral load <1000 HIV RNA copies/mL at Month 12 who were matched according to gender, age, CD4+ T cell count and viral load at baseline.

The characteristics of patients with and without virological failure were compared using the Wilcoxon rank sum test for continuous data and χ^2 tests for categorical data.

Risk factors were assessed by logistic regression models. All the tests were two-sided and $P<0.05$ was considered significant. STATA v12 was used for all analyses. One patient with HIV group O virus, which is naturally resistant to NNRTI drugs, was excluded from the risk factor analysis.

Results

A total of 300 HIV-positive patients who started ART were included from January 2010 to October 2010. Viral load testing at Month 12 was successfully performed for 238 patients (Figure S1) and these patients were included in the analyses. Of these patients, 200 (84%) had a viral load of <1000 HIV RNA copies/mL, 160 (67%) patients had a viral load of <50 HIV RNA copies/mL and 38 (16%) patients experienced virological failure (Table 1).

At least one HIV-1 drug-resistance mutation was detected in 27/38 (71%) patients with virological failure at Month 12 using population sequencing. In 24 patients (63%), mutations associated with high-level resistance to any of the prescribed drugs were found. The M184V mutation was the most frequently detected NRTI mutation ($n=18$), the most commonly observed NNRTI resistance mutations being K103N ($n=10$), Y181C ($n=7$) and G190A ($n=6$) (Table S1).

Drug-resistance mutations at baseline were detected in 6/30 patients (20%) experiencing virological failure and 6/35 (17%) matched controls using next-generation sequencing ($P=0.77$). The most common mutations were V90I ($n=4$) and V108I ($n=3$). The M184V mutation was detected in one patient at a frequency of 1.3% and the K103N mutation was detected in another patient at a frequency of 1.7%. Both patients had virological failure at Month 12, when neither the M184V nor the K103N mutation was detected (Table S1).

In the multivariable logistic regression, a lower baseline CD4 cell count (OR per 100 cells/mm^3 lower 1.47, 95% CI 1.02–2.08, $P=0.04$) and a lower adherence ratio (OR per 1% lower 1.04, 95% CI 1.02–1.07, $P<0.001$) were associated with a higher risk of virological failure (Table 2). A higher age was associated with a higher adherence ratio ($P=0.01$) but not with lower virological failure rates ($P=0.21$). The most common reasons for incomplete drug intake (adherence ratio <95%) included 'drugs were out of stock' (37%), 'patient was out of town and could not collect drugs in time' (8%) and 'discontinued drugs because of side effects' (8%).

Discussion

In this study in rural Cameroon, 67% of patients achieved a viral load of <50 HIV RNA copies/mL after 1 year of treatment. HIV-1 drug-resistance mutations were detected in 71% of patients with virological failure. A previous study from the capital of Cameroon reported an undetectable viral load in 49% of patients after 2 years of ART, with major drug mutations being detected in only 10%.[8] Being treated in a rural centre has previously been shown to be a risk factor for virological failure in a study from Gabon.[9]

Poorer adherence was clearly associated with a higher risk of virological failure. Interestingly, younger patients (<36 years) showed poorer adherence but similar rates of virological failure and resistance compared with older patients. This supports previous findings that good adherence does not protect from the development of drug resistance if the drugs are continued in the absence of complete viral suppression.[10]

The predominant reasons for non-adherence were organizational; 41% of patients stated that their refill date was scheduled on a public holiday or that the ART drugs were out of stock. This suggests that repeated interruptions of drug supply and subsequent intake of antiretroviral drugs may be a specific problem in rural Africa. Our results are in line with those from studies from South Africa and Malawi suggesting that incomplete adherence is associated with a higher risk of virological failure[11] and that patients with previous treatment interruptions have higher rates of drug resistance.[12]

Using next-generation sequencing with a detection limit of 1%, we found primary drug-resistance mutations prior to ART initiation in 20% of patients with later virological failure, and at a

similar frequency in matched controls without virological failure. None of the mutations occurred at a frequency high enough to be picked up by conventional population-based sequencing. A previous pooled analysis shows an association between the existence of minority drug-resistance mutations and virological failure,[13] but the clinical use of detecting minorities in longitudinal studies seems, so far, to be limited.[14–16] We found that acquired drug-resistance mutations became detectable within the first year of antiretroviral therapy in a substantial number of patients. Because of cross-class resistance, future therapeutic options are limited to drugs from other classes such as protease or integrase inhibitors, which are not yet widely available in Africa.[17]

Our study had the limitation that follow-up was limited to 1 year and the number of patients with virological failure was limited. Minority variants were only assessed in a subset of patients with virological failure and matched controls. However, our study is among the very few to provide information on the existence of minority variants in an African setting.

In conclusion, minority drug-resistance mutations were detected by next-generation sequencing at baseline in a substantial proportion of patients but were not associated with virological failure after 1 year of first-line ART. An important reason for incomplete adherence to ART, as a major determinant of the substantial virological failure rates in our study, was the unavailability of drugs at the treatment centre. Strategies for an uninterrupted supply chain to rural parts in Africa seem pivotal in order to achieve sustainable treatment success.

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Transparency declarations

None to declare.

Supplementary data

Supplementary data, including Figure S1 and Table S1, are available at *JAC* Online (http://jac.oxfordjournals.org/).

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